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Comparative Effects of Aspirin, a Synthetic Thrombin Inhibitor and a Monoclonal Antiplatelet Glycoprotein IIb/IIIa Antibody on Coronary Artery Reperfusion, Reocclusion and Bleeding With Recombinant Tissue-Type Plasminogen Activator in a Canine Preparation

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The comparative effects of intravenous aspirin, the synthetic thrombin inhibitor (2R,4R)-4-methyl-1-[N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidinecarboxylic acid monohydrate (Argatroban) and F(ab')2 fragments of monoclonal antibody 7E3 against platelet glycoprotein IIb/IIIa (7E3-F[ab']2) on thrombolysis, reocclusion and bleeding associated with 0.45 mg/kg body weight bolus injections of recombinant tissue-type plasminogen activator (rt-PA) were studied in a canine coronary artery thrombosis model. Coronary patency was monitored for 2 h both by flow probe and by coronary angiography.

Four groups were studied: Group I = pretreated with 17 mg/kg intravenous aspirin (n = 6), Group II = pretreated with 200 μg/kg per min intravenous Argatroban for 60 min (n = 5), Group III = pretreated with aspirin and Argatroban (n = 5) and Group IV = pretreated with 0.8 mg/kg intravenous 7E3-F(ab')2 (n = 5). In Group I, reflow occurred in four of six dogs, but did not persist; reflow was induced in Group II in four of five dogs, persisting in one; in Group III, reflow occurred in all five dogs, persisting in four; in Group IV reflow was achieved in four of five dogs, persisting in two.

The frequency of persistent reflow in Group III was significantly higher than in the combined Groups I and II (p = 0.012), whereas the time to reflow was significantly shorter in the groups receiving Argatroban than in the aspirin group (median 25 versus 55 min, p = 0.04). There were no significant differences between Groups III and IV. Bleeding times prolonged from 4.2 ± 1.2 min to 5 ± 3 min at 60 min in Group I, to 9.4 ± 11 min in Group II, to 12 ± 11 min in Group III and to 27 ± 8 min in Group IV. One hour after the end of infusion, the bleeding times were 5.0 ± 0.9, 5.2 ± 1.3, 14 ± 9.2 and 26 ± 9 min, respectively.

These findings indicate that Argatroban accelerates coronary thrombolysis with rt-PA and that the combination of aspirin and Argatroban prevents coronary reocclusion. This is achieved in association with less pronounced prolongation of the bleeding time than observed with the potent antiplatelet glycoprotein IIb/IIIa receptor antibody. Inhibition of platelet activation with aspirin in combination with a short-lived synthetic thrombin inhibitor may constitute an alternative approach to improve coronary artery patency with thrombolytic therapy in patients with acute myocardial infarction.

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Recanalization of the occluded coronary artery with thrombolytic therapy may reduce the mortality rate in patients with acute myocardial infarction (1–4), but the effect is most pronounced if reflow is obtained early and is persistent. However, with the currently used agents and infusion protocols, coronary artery reflow is often delayed (5–7) and reocclusion is relatively frequent (8–12). Consequently, different therapeutic approaches have been investigated to accelerate recanalization and prevent reocclusion. These include the use of anticoagulants (13,14) and potent anti-

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platelet agents (15–18). A monoclonal antibody against the platelet glycoprotein IIb/IIIa receptor has been shown to accelerate coronary thrombolysis (19,20) but in association with marked prolongation of the bleeding time (19). Alternatively, selective thrombin inhibitors including tripeptide chloromethyl ketone (21), recombinant hirudin (22) and (2R,4R)-4-methyl-1-[N^2-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidinecarboxylic acid monohydrate (Argatroban) (23) have been shown to prevent platelet-mediated arterial thrombosis.

In a recent study (24) in a rabbit model of femoral artery thrombosis, we compared the effects of aspirin, heparin and Argatroban on the speed of thrombolysis with those of recombinant tissue-type plasminogen activator (rt-PA) on reocclusion after initial lysis and on the bleeding time. Aspirin in combination with rt-PA was found to prolong the bleeding time, but did not accelerate reflow or prevent reocclusion. Argatroban was significantly more potent than heparin for the acceleration of recanalization and the prevention of reocclusion, but it also prolonged the bleeding time. Aspirin potentiated the effect of Argatroban on bleeding time but did not significantly potentiate its effect on the speed of lysis or the frequency of reocclusion.

Therefore, we have investigated the comparative effects of aspirin, Argatroban and their combination on thrombolysis, reocclusion and bleeding in a canine model of coronary artery thrombosis with superimposed high grade stenosis (25) that has many anatomic similarities to the coronary artery occlusion in patients with acute myocardial infarction. In this model it was also possible to directly compare the efficacy and tendency to cause bleeding of these antithrombotic strategies with those of the murine monoclonal antibody 7E3, a very potent antiplatelet agent with an established beneficial effect on thrombolysis and reocclusion with rt-PA.

Methods

Canine coronary artery thrombosis model with superimposed endothelial cell damage and high grade stenosis. The experimental model was used as described elsewhere (25), with some modifications. Adult mongrel dogs (20 to 25 kg in weight) were anesthetized with pentobarbital (30 mg/kg body weight intravenously) and additional doses as required. The dogs were intubated and placed on a respirator with a tidal volume of 10 to 15 ml/kg. Procainamide (1.5 g intramuscularly) and lidocaine (0.1 mg/kg per min intravenously) were given for prophylaxis of arrhythmias. The left carotid artery was exposed through an incision in the neck and cannulated with a number 7-1 modified Amplatz coronary angiographic catheter. Thoracotomy was performed through the left fifth intercostal space, with cannulation of the left internal mammary artery for continuous blood pressure recording. The pericardium was opened and suspended to create a pericardial cradle. The left anterior descending coronary artery was dissected from the epicardium and a 2.5 cm segment was isolated distal to the first diagonal branch. A 0.7 mm internal diameter catheter was inserted into a side branch of the isolated left anterior descending coronary artery segment and an ultrasonic flow probe (T101 Transsonic System) was placed on the proximal portion of the artery for continuous blood flow monitoring. Selective angiography of the left anterior descending coronary artery was obtained with injection of 1 to 2 ml meglumine diatrizoate and with videotape recording. One milliliter of blood was drawn for thrombus formation.

The dog was then given heparin (5mn), either subcutaneously (1,000 U injected into each of two separate sites in the back) in Groups I to III or intravenously (bolus injection of 4,000 and 1,000 U/h) in Group IV. A 2 mm wide plastic wire tie (Mass. Gas and Electric Supply) was progressively constricted around the left anterior descending coronary artery immediately distal to the proposed site of thrombus formation to limit the blood flow to 40 ± 10% of the baseline value. A control angiogram was then performed.

The isolated left anterior descending coronary artery segment was traumatized by four consecutive external compressions with blunt forceps for 3 to 5 s to damage the endothelium and promote thrombus adherence. Snare occlusions were made distal to the flow probe and proximal to the constriction site. Thrombin (0.1 ml of 100 U/ml, Thrombinar, Armour Pharmaceutical) mixed with 0.3 ml of blood was injected through the side branch catheter into the emptied coronary artery segment. After 5 min, the proximal snare was released, and 2 min later, the distal tourniquet. The side branch catheter was removed, but the permanent constrictor remained in place. An angiogram was performed 30 min after thrombus formation to confirm total occlusion of the artery, as demonstrated by the ultrasonic flow probe.

Infusion protocols and evaluation of coronary artery patency. Four groups of five or six dogs were studied: Group I with intravenous bolus injection of 17 mg/kg aspirin (Synthelabo Benelux); Group II with intravenous infusion of 200 μg/kg per min Argatroban (Genentech) over 60 min; Group III with administration of the combination of aspirin and Argatroban and Group IV with intravenous injection of 0.8 mg/kg 7E3-F(ab')2 (Centocor) 5 min after neutralization of circulating heparin in this group with intravenous injection of 10 mg protamine sulfate. The timing of the surgical procedure and infusion protocol is represented in Figure 1, with a time scale adjusted to the first bolus injection of rt-PA (Activase, Genentech). In all groups, the partial thromboplastin time was measured before beginning the study infusion. Intravenous administration of these study drugs was carried out with a constant rate Harvard infusion pump and started after the confirmation of stable complete occlusion for 30 min by coronary angiography. Ten minutes later, rt-PA was given as an intravenous bolus injection of 0.45

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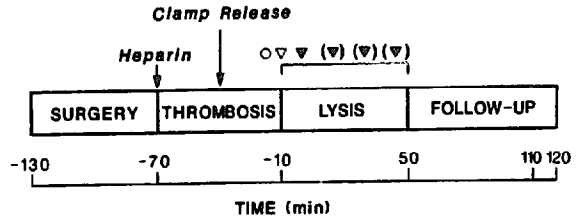

Figure 1. Schematic representation of the time course of the experiments. The surgical procedure for the production of a whole blood clot with superimposed endothelial cell damage and high grade stenosis was carried out over approximately 90 min. The clot was aged for 30 min before bolus administration of aspirin or 7E3-F(ab')2 (**open triangle**) or the start of the Argatroban infusion (⌐¬). Ten minutes later a bolus injection of 0.45 mg/kg of recombinant tissue-type plasminogen activator (rt-PA) (**closed triangles**) was given and repeated at 15 min intervals until reperfusion occurred or until a maximum of four bolus injections. The coronary patency status was monitored continuously for 120 min after the first rt-PA bolus injection. Blood samples were taken at baseline, before the administration of study drug (aspirin, 7E3-F[ab']2 or Argatroban), 20 min after the first rt-PA injection and toward the end of the experiment. O = protamine sulfate injection (in 7E3-F(ab')2 group only).

mg/kg at 15 min intervals until recanalization of the thrombosed coronary artery was achieved or up to a maximum of four bolus injections. A coronary angiogram was obtained every 15 min to monitor occlusion and when the flow probe showed evidence of reflow. Reflow was monitored both angiographically and by flow probe for at least 2 h after the initial angiographic confirmation of stable coronary occlusion.

The reflow time was recorded as the time from the first rt-PA bolus injection until recanalization was documented by the return of blood flow in the artery to ≥25% of that before thrombus formation and confirmed by complete angiographic filling of the apex with rapid clearance of the dye in four or fewer heartbeats. After recanalization was obtained, blood flow was monitored for evidence of reocclusion, defined as <25% baseline flow, with the final confirmation obtained by angiography showing dye clearance in more than five cycles. The reocclusion time was defined as the interval between documented reflow and reocclusion. Frequently, cyclic reflow occurred, interspersed with periods of reocclusion.

The coronary artery patency status was categorized as follows: 1) persistent occlusion = no reflow; 2) reocclusion after reflow = reocclusion persisting for ≥60 min before the end of the experiment, after initial reflow; 3) cyclic reflow = alternating reocclusion and recanalization after initial reflow; and 4) persistent patency = persistent flow without reocclusion after initial reflow. The studies with experimental animals were carried out in conformance with the Position of the American Heart Association on Research Animal Use, adopted November 11, 1984.

Hemostasis assays. Blood samples were taken at baseline, before the administration of aspirin, Argatroban or 7E3-F(ab')2, 20 min after the first rt-PA bolus injection and toward the end of the experiment. Blood samples for determination of the activated partial thromboplastin time, thrombin time and glycoprotein IIb/IIIa receptor saturation were drawn into a 0.01 *M* citrate solution.

The binding of iodine-125–7E3 to platelets was measured by incubating 0.2 ml citrated platelet-rich plasma at 3.0 ± 0.1 × 10^8 platelets/ml with 20 μl of iodine-125–labeled 7E3 at a final concentration of 20 μg/ml, which was previously shown to be a near saturating dose (26). After 55 min at 22°C, duplicate 0.1 ml aliquots of the samples were layered over 0.1 ml 30% sucrose solution contained in 400 μl microcentrifuge tubes and centrifuged at 12,000 *g* for 5 min at 22°C. The radioactivity in both the platelet pellet and the supernatant fluid was then determined and the total number of molecules of bound iodine-125–7E3 was calculated from the antibody added, the percent of radioactivity in the pellet and the platelet count. The number of glycoprotein IIb/IIIa receptors blocked by in vivo infusion of unlabeled 7E3-F(ab')2 was defined as the reduction in the number of iodine-125–7E3 molecules that could bind per platelet after the infusion.

Samples for rt-PA and fibrinogen determination were collected in 0.01 *M* of citrate and aprotinin (200 kallikrein inhibitor units/ml, Sigma Chemical) solution. Fibrinogen was measured by the coagulation rate assay of Clauss (27) as modified by Vermylen et al. (28). This assay is insensitive to heparin in plasma concentrations of up to 10 U/ml (29). The rt-PA antigen was measured by enzyme-linked immunosorbent assay (ELISA) as described elsewhere (30). Platelet aggregation with 1 μl epinephrine (0.5 μ*M*) and 14 μl arachidonic acid (0.5 μ*M*) (31) or with 55 μl adenosine diphosphate (ADP) (100 μ*M*) (32) per 450 μl platelet-rich plasma was performed as described elsewhere.

Template bleeding times were performed on the shaved left medial foreleg using a spring-loaded blade device (Surgicutt International Technidyne).

Pathologic examination. At the end of the experiment, the dogs were killed with an overdose of pentobarbital. The heart was removed, recanalized arteries were perfusion fixed in situ (33) and the whole heart was fixed in a 5% formaldehyde solution. The thrombosed, stenotic and poststenotic segments of the left anterior descending coronary artery were then removed intact, embedded in paraffin blocks and sectioned longitudinally. Longitudinal sections were stained with hematoxylin-eosin and examined microscopically for the presence of intraluminal or mural thrombi. The relative amount of platelets and red blood cells in the thrombus was evaluated.

Statistical analysis. Values are reported as mean values ± SD. The significance of differences between groups was determined with Student's *t* test for paired or unpaired

Table 1. Effects of Aspirin, Argatroban and 7E3-F(ab')2 on Coronary Artery Recanalization and Reocclusion With Recombinant Tissue-Type Plasminogen Activator

Experimental Group	No.	Blood Flow (ml/min)		Coronary Patency* (frequency)				Reperfusion†			
									Time (min)		
		Baseline	Poststenotic	PO	RR	CR	PP	Total No.	Mean ± SD	Median	Range
I: aspirin	6	16 ± 5	6.3 ± 1.1	2	2	2	0	4/6	40 ± 20	55	14–120
II: Argatroban	5	15 ± 3	6.0 ± 1.7	1	0	3	1‡	4/5	23 ± 14	28	7–120
III: aspirin + Argatroban	5	12 ± 4	5.1 ± 0.5	0	0	1	4§	5/5	24 ± 11	21	14–39
IV: 7E3-F(ab')2	5	13 ± 5	5.0 ± 1.8	1	1	1	2‖	4/5	27 ± 17	33	9–120

*p = 0.036 for significant differences by Kruskal-Wallis nonparametric analysis of variance; †data are mean values ± SD of values in responsive animals, and the median and range are values for the total group; ‡p = NS versus aspirin; §p = 0.009 versus pooled aspirin and Argatroban groups, p = 0.064 versus Argatroban and p = 0.008 versus aspirin; ‖p = NS versus pooled aspirin and Argatroban and versus aspirin + Argatroban. Unless indicated, data are mean values ± SD. CR = cyclic reflow; PO = persistent occlusion; PP = persistent patency; RR = reflow and reocclusion.

values. A Kruskal-Wallis nonparametric analysis of variance was performed (34) on ranks of the ordered variable of arterial patency, which ranged from 0 = persistent occlusion, 1 = reflow with reocclusion, 2 = cyclic reflow and reocclusion and 3 = persistent patency. This form of analysis of variance was selected because of the non-Gaussian distribution of the patency state variable. Fisher's exact test was used to compare the occurrence of reflow and reocclusion in the various groups.

Results

Coronary artery reflow and reocclusion. The effects of the different infusion protocols on coronary patency status, categorized as persistent occlusion, reocclusion after initial reflow, cyclic reflow and reocclusion and persistent patency are summarized in Table 1. The patency status in the individual dogs is also schematically represented in Figure 2. The external constrictor reduced blood flow on average to 40% of the baseline level, from a mean value of 14 to 5.6 ml/min, before thrombus formation. Bolus injection of rt-PA in Group I dogs given intravenous aspirin induced recanalization in four of the six dogs (Fig. 2, Dogs 2, 4, 5 and 6). Reflow was achieved in these four dogs after a mean time of 40 ± 20 min, (median time of 55 min, range 14 to 120 in the six dogs) (Table 1). Reocclusion occurred in all four dogs within 3 ± 1 min and was persistent in two (Fig. 2, Dogs 2 and 4) and cyclic in the other two (Dogs 5 and 6). The median value of the number of rt-PA bolus injections was four, with a range of one to four (Fig. 2).

Recombinant tissue-type plasminogen activator combined with Argatroban (Group II) induced reflow in four of

Figure 2. Schematic representation of the coronary artery patency status in dogs receiving 0.45 mg/kg bolus injections of recombinant tissue-type plasminogen activator (rt-PA) in association with administration of aspirin, Argatroban or 7E3-F(ab')2. ═══ = continuous intravenous infusion of Argatroban. **Open bars** represent patency and **hatched bars** occlusion of the coronary artery. The **half-open bar** represents partial reflow. Other symbols as in Figure 1.

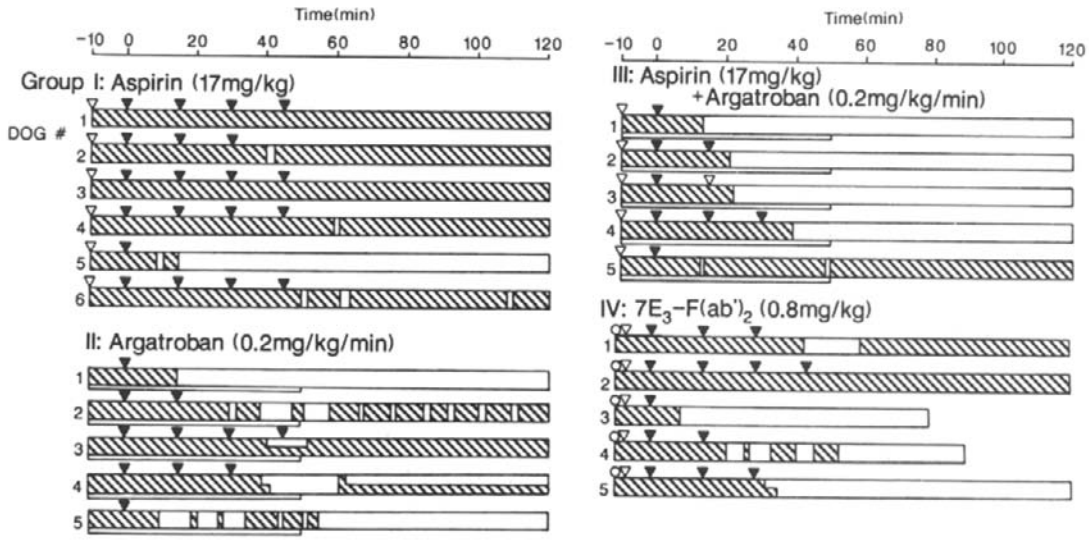

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Table 2. Effect of Aspirin, Argatroban and 7E3-F(ab')2 on Template Bleeding Time Induced With Recombinant Tissue-Type Plasminogen Activator (rt-PA)

Experimental Group	No.	Before	20 Min After First rt-PA Bolus	60 Min After Last rt-PA Bolus	End
I: aspirin	6	3.8 ± 0.9	5.3 ± 0.5	5.0 ± 0.9	5.0 ± 0.9
II: Argatroban	5	3.8 ± 0.8	9.4 ± 11	5.2 ± 1.3	5.2 ± 1.0
III: aspirin + Argatroban	5	4.2 ± 1.1	12 ± 11	10 ± 3	14 ± 9.2
IV: 7E3-F(ab')2	5	5 ± 3	27 ± 8	—	26 ± 9

Data are mean values ± SD; results >30 min are given a 30 min value for calculation of the mean value ± SD.

five dogs (Fig. 2, Dogs 1, 2, 4 and 5), with a mean time to recanalization of 23 ± 14 min (median 28, range 7 to 120). In three of these four dogs (Dogs 2, 4 and 5), the artery reoccluded within 11 ± 13 min and demonstrated cyclic changes throughout the experiment, whereas in one dog (Dog 1) it remained patent. The median number of rt-PA bolus injections was two, with a range of one to four.

Group III, treated with aspirin and Argatroban in combination with rt-PA, showed recanalization in all six dogs, with an average time to reflow of 24 ± 11 min, (median 21, range 14 to 39) (Table 1). Reocclusion occurred in one dog (Dog 5) within 13 min, whereas persistent patency was observed in the other four. The median number of rt-PA bolus injections was two, with a range of one to four. *In Group IV treated with 7E3-F(ab')2 and rt-PA*, recanalization was achieved in four of five dogs, with a time to reflow of 27 ± 17 min (median 33, range 9 to 120). Reocclusion was observed in one dog (Dog 1) and cyclic reflow in another (Dog 4), with a time to reocclusion of 11 ± 9 min, whereas the other two dogs (Dogs 3 and 5) showed persistent patency. The median number of rt-PA bolus injections was three (range one to four).

Several significant differences in coronary artery patency were observed with the various infusion protocols. A Kruskal-Wallis analysis of all experiments with aspirin, Argatroban and a combination of the two, with patency status ordered in the sequence of persistent occlusion, reocclusion, cyclic reflow and persistent patency, yielded a p value of 0.036. Comparison of Argatroban with aspirin resulted in a p value of 0.18; comparison of the combination of aspirin and Argatroban with aspirin showed p = 0.008 and that of Argatroban plus aspirin versus Argatroban p = 0.064. Analysis of the patency status of the combination of aspirin and Argatroban versus the pooled data of either agent alone resulted in a p value of 0.009. Persistent patency occurred more frequently with the combination of aspirin and Argatroban than with either agent alone (4 of 5 versus 1 of 11, p = 0.013 by Fisher's exact test). Kruskal-Wallis analysis of the patency data obtained with 7E3-F(ab')2 (Group IV) versus the combined Groups I and II treated with either aspirin or Argatroban yielded p = NS, whereas comparison with Group III treated with the combination resulted in p = NS.

When the dogs were categorized by time to reflow of > or <40 min, two of six dogs in the aspirin group, four of five in the Argatroban group and five of five in the combination group fell into the category of <40 min (p = 0.06). Comparison of the results in the aspirin group with the pooled data from the Argatroban groups yielded p = 0.036, the same p value obtained when the number of rt-PA bolus injections was categorized into four or fewer than four.

Hemostatic analysis. Before administration of the study agent, the activated partial thromboplastin time was normal in all animals. Serial template bleeding times were measured in all dogs and the results are summarized in Table 2. Aspirin in combination with rt-PA prolonged the bleeding time slightly but significantly from 3.8 ± 0.9 to 5.3 ± 0.5 min (p = 0.03). Argatroban plus rt-PA induced prolongation of the bleeding time from 3.8 ± 0.8 to 9.4 ± 11 min (p = 0.14), but this was rapidly reversible. However, when these agents (rt-PA, aspirin and Argatroban) were combined, the bleeding was significantly prolonged from 4.2 ± 1.1 to 12 ± 11 min (p = 0.04) and this prolongation persisted throughout the experiment. When 7E3-F(ab')2 was combined with rt-PA, the bleeding time was prolonged from 5 ± 3 to 27 ± 8 min (p = 0.0004), which persisted throughout the experiment.

Table 3 summarizes the results of platelet function tests. Platelet counts did not change markedly in any of the experimental groups. Epinephrine/arachidonic acid aggregation was totally abolished in the groups receiving aspirin (data not shown), whereas ADP-induced platelet aggregation was abolished in the group receiving 7E3-F(ab')2. Injection of 7E3-F(ab')2 produced >75% blocking of glycoprotein IIb/IIIa receptors (81 ± 13% at 30 min and 76 ± 22% at the end of the experiment) (data not shown).

Table 3 also summarizes the results of activated partial thromboplastin and thrombin times. Aspirin in combination with rt-PA did not affect the activated partial thromboplastin or thrombin times before administration of rt-PA, whereas a moderate prolongation of the results of both assays was observed at the end of the experiment. This could be ascribed to fibrinogen depletion in two of the animals given four bolus injections of rt-PA. Argatroban at a dose of 200 μg/kg per min for 60 min prolonged the activated partial thromboplastin time from 13 ± 1 to 80 ± 28 s and the

Table 3. Effect of Aspirin, Argatroban and 7E3-F(ab')2 on Platelet Function and Hemostatic Variables

Experimental Group	No.	Platelet Count ($\times 10^3$/mm^3)		Platelet Aggregation (T%)		Activated Partial Thromboplastin Time (s)			Thrombin Time (s)		Fibrinogen Level (g/liter)	
		Pre	End	Pre	End	Baseline	Before rt-PA	End	Baseline	End	Baseline	End
I: aspirin	6	310 ± 75	280 ± 50	70 ± 11	34 ± 18	11 ± 1	11 ± 1	47 ± 43	14 ± 1	60 ± 40	1.7 ± 0.2	0.6 ± 0.7
II: Argatroban	5	340 ± 40	260 ± 70	66 ± 9	58 ± 16	13 ± 1	80 ± 28	35 ± 37	17 ± 7	90 ± 21	1.9 ± 0.1	0.9 ± 0.6
III: aspirin + Argatroban	5	350 ± 70	250 ± 60	64 ± 9	45 ± 6	12 ± 2	91 ± 21	37 ± 36	13 ± 4	>100	1.9 ± 0.6	1.1 ± 0.3
IV: 7E3-F(ab')2	5	150 ± 90*	230 ± 100	50 ± 30	0	16 ± 3 18 ± 7†	96 ± 9	20 ± 4	14 ± 3	45 ± 31	2.4 ± 1.1	0.3 ± 0.4

*After protamine sulfate injection; †after neutralization of circulating heparin with protamine sulfate. Data are mean values ± SD. rt-PA = recombinant tissue-type plasminogen activator; T% = percent change in light transmission.

thrombin time from 17 ± 7 to 90 ± 21 s when assayed before the first rt-PA bolus injection. At the end of the experiment, both assay values were still prolonged, but this could not be explained by fibrinogen depletion in the one animal given four bolus injections of rt-PA. With the combination of aspirin and Argatroban, similar changes were observed. In the group given 7E3-F(ab')2, the partial thromboplastin time was prolonged after heparin administration, but normalized after protamine sulfate injection.

The results of plasma fibrinogen assays are shown in Table 3. Changes in fibrinogen levels correlated with the number of rt-PA bolus injections given. In five dogs that received four bolus injections of rt-PA, irrespective of the infusion protocol, fibrinogen decreased to 16 ± 35% of the baseline value at the end of the experiment; in five dogs given three bolus injections of rt-PA, the residual fibrinogen level decreased by 33 ± 28%; in four with two bolus injections, it decreased by 46 ± 20% and in four receiving one bolus injection of rt-PA, the residual fibrinogen level at the end of the experiment was 60 ± 40% of the baseline value. The rt-PA antigen level measured at 1 min after bolus injection of 0.45 mg/kg increased to 5.3 ± 0.5 μg/ml and decreased to 0.72 ± 0.14 μg/ml 10 min after the injection (data not shown). The hematocrit remained stable (41 ± 7%) in the aspirin-treated dogs in which 530 ± 470 ml fluid was infused during the experiment (data not shown). In the other groups, the hematocrit decreased from 43% to 46% before to 30% to 35% at the end of the experiment and the volume of liquid transfused ranged from 1,100 to 1,800 μl. Thus, the amount of infused saline solution was higher in the groups receiving Argatroban or 7E3-F(ab')2 than in the group receiving aspirin alone, notwithstanding the fact that more rt-PA bolus injections were given in the aspirin group.

Pathologic examination (Table 4). The extent of arterial thrombosis was graded as occlusive thrombus, partially occlusive thrombus, mural thrombus and patent artery and the composition of the thrombus as platelet rich, erythrocyte rich or mixed with interlaced platelet-rich and erythrocyte-rich thrombus.

Light microscopic examination (Fig. 3) revealed that segments not recanalized and segments with reocclusion after initial reflow contained occlusive thrombus composed primarily of red blood cell clots (Table 4), whereas segments with persistent patency contained either platelet-rich mural thrombus or no significant thrombus. All segments showed significant mural disruption and intramural hemorrhage. Patent segments often showed prominent leukocyte margination overlying the damaged media.

Table 4. Results of Pathologic Analysis of Thrombosed Left Anterior Descending Coronary Arteries

Experimental Group	Dog No.	Description
I: aspirin	1	OT, ER
	2	MT, PR
	3	OT, ER
	4	OT, ER
	5	PA
	6	OT, ER
II: Argatroban	1	MT, PR
	2	OT, ER
	3	MT, PR
	4	OT, MPE
	5	—
III: aspirin + Argatroban	1	PT, PR
	2	MT, PR
	3	MT, PR
	4	MT, PR
	5	MT, MPE
IV: 7E3-F(ab')2	1	OT, MPE
	2	OT, MPE
	3	MT, ER
	4	MT, ER
	5	MT, ER

Arterial thrombosis is graded as: MT = mural thrombus; OT = occlusive thrombus; PA = patent artery; PT = partially occlusive thrombus. Thrombus composition is graded as: ER = erythrocyte rich; MPE = mixed with interlaced platelet-rich and erythrocyte-rich thrombus; PR = platelet rich.

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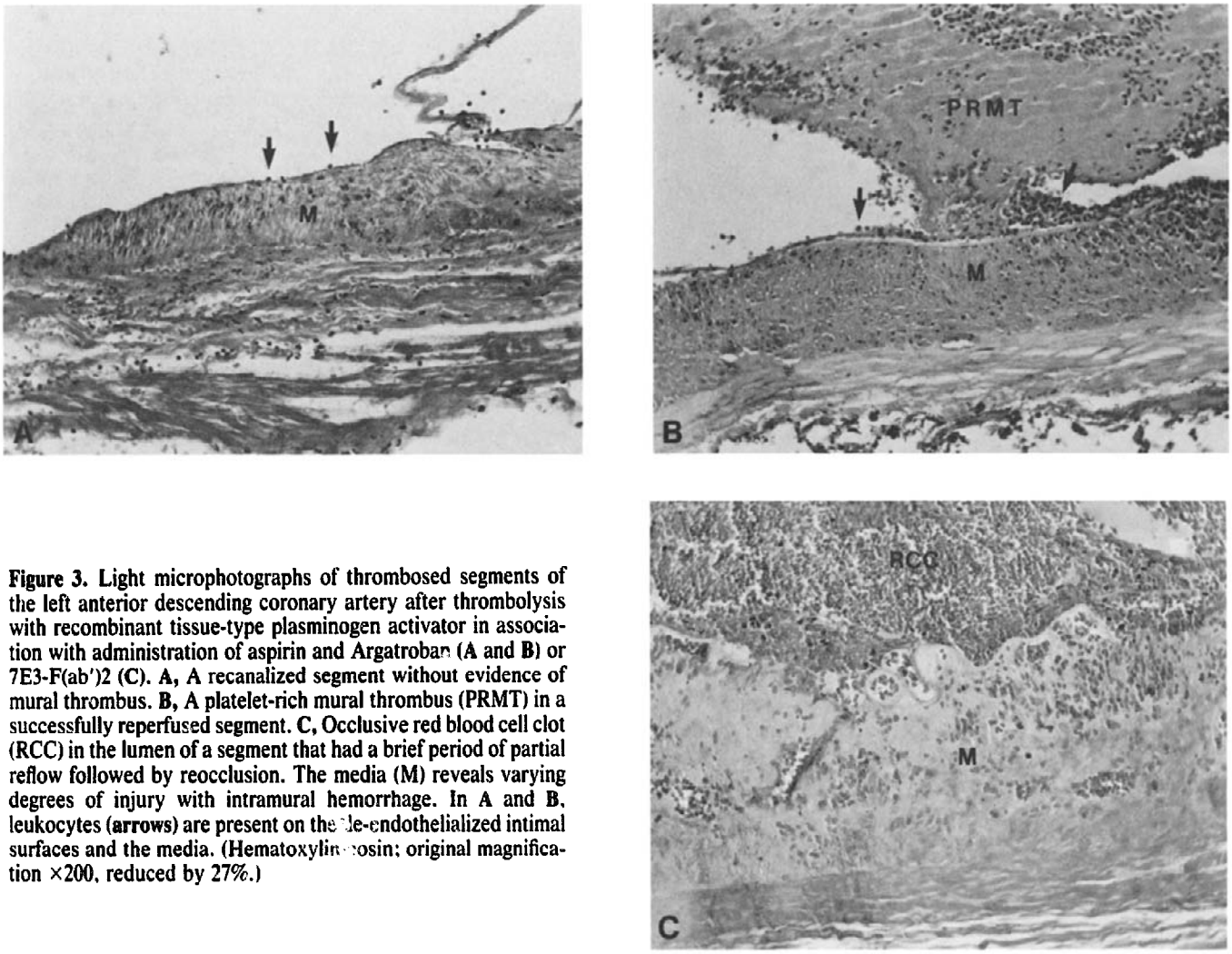

Figure 3. Light microphotographs of thrombosed segments of the left anterior descending coronary artery after thrombolysis with recombinant tissue-type plasminogen activator in association with administration of aspirin and Argatroban (**A** and **B**) or 7E3-F(ab')2 (**C**). **A,** A recanalized segment without evidence of mural thrombus. **B,** A platelet-rich mural thrombus (PRMT) in a successfully reperfused segment. **C,** Occlusive red blood cell clot (RCC) in the lumen of a segment that had a brief period of partial reflow followed by reocclusion. The media (M) reveals varying degrees of injury with intramural hemorrhage. In **A** and **B**, leukocytes (**arrows**) are present on the de-endothelialized intimal surfaces and the media. (Hematoxylin-eosin; original magnification ×200, reduced by 27%.)

Discussion

Thrombolytic therapy with recombinant tissue-type plasminogen activator (rt-PA), one of the most potent agents for coronary artery recanalization in patients with acute myocardial infarction (35), still suffers from significant shortcomings. When rt-PA is used alone or in combination with heparin anticoagulation, coronary reocclusion and unstable ischemic events occur in 5% to 30% of patients and a few patients remain at risk for serious bleeding (36).

Role of platelets in coronary thrombosis and thrombolysis. Platelets appear to play a central role in the current limitations of thrombolytic therapy. Coronary artery thrombosis is initiated by platelet activation and aggregation at the site of ruptured atherosclerotic plaque (37–39). The mechanism of platelet-mediated thrombosis is complex, involving platelet adhesion and aggregation, as well as activation of the coagulation system by the exposed subendothelial structures. In both animal models (25) and patients (6), treatment with aspirin in combination with heparin is not uniformly effective in preventing thrombus formation at the site of vascular injury. Two alternative approaches have been successful in preventing reocclusion after thrombolysis with rt-PA, namely, maintenance rt-PA infusion (12) or the use of a potent antiplatelet glycoprotein IIb/IIIa antibody (19). However, both agents increase the bleeding time and increase the risk of bleeding (19,40).

Recent studies by us (23,24) and others (21,22,41) have indicated that selective thrombin inhibitors may be effective in the prevention of platelet-mediated arterial thrombosis. Therefore, we sought to determine whether acceleration of thrombolysis and prevention of reocclusion require extensive inhibition of platelet aggregation, such as with the antiplatelet glycoprotein IIb/IIIa 7E3 antibody (19) or combined thromboxane A_2 and serotonin S_2 receptor antagonists (18), or whether it is possible to achieve comparable efficacy with the use of a potent but short-lived synthetic thrombin inhibitor.

Interpretation of the results of the present study. We used a canine model of coronary artery thrombosis with superimposed high grade stenosis, which has many anatomic similarities to the coronary artery occlusion in patients with

acute myocardial infarction (25). In this model, the effects of Argatroban on the speed of thrombolysis with rt-PA, on reocclusion and on the bleeding tendency as revealed by the template bleeding time were studied and compared with the effects of aspirin and of the monoclonal antiplatelet glycoprotein IIb/IIIa antibody. Interference of systemic therapeutic heparin anticoagulant therapy was avoided by subcutaneous administration of a low dose of heparin that did not prolong the activated partial thromboplastin or thrombin times (the prolongation toward the end of the infusion in the aspirin group being the result of excessive fibrinogen breakdown in dogs that received four bolus infusions of rt-PA). Alternatively, intravenously injected heparin in the 7E3-F(ab')2 group was reversed with protamine sulfate. The entire blood clot in the left anterior descending coronary artery was produced with 10 U of thrombin per 0.3 ml of blood. However, the dogs were given heparin to prevent extension of the clot after clamp release and the clot was aged for 30 min before initiation of the infusion protocol. This time course was considered to be sufficient for the total inhibition of remaining thrombin before the administration of study drugs.

Intravenous bolus injection of 17 mg/kg aspirin completely abolished platelet aggregation induced with epinephrine and arachidonic acid, but in combination with bolus injections of rt-PA only marginally prolonged the template bleeding time. This observation was unexpected because we previously observed a marked interactive effect on the bleeding time prolongation by perorally administered aspirin in combination with a continuous infusion of rt-PA in nonheparinized dogs (Garabedian et al., unpublished observations) as well as of intravenously administered aspirin in combination with rt-PA bolus injection in rabbits (24). Our present observations cannot be explained by variations in technique because the effect of the glycoprotein IIb/IIIa antibody on the bleeding time in the present study was very similar to that observed in our previous study (19). Clearly, the variable response of different administration schemes of rt-PA in aspirin-treated dogs warrants further investigation because it might result in a therapeutic regimen with a reduced bleeding tendency in human patients.

Intravenous bolus injection of aspirin did not markedly potentiate the efficacy of rt-PA in coronary artery thrombolysis as revealed by both our present results and the results of our previous study (25). Short-term infusion of Argatroban in a dose that prolonged the activated partial thromboplastin time from approximately 15 to 85 s accelerated thrombolysis compared with the group receiving aspirin alone, whereas the combination of Argatroban and aspirin prevented reocclusion relative to the groups treated with either drug alone. In the groups receiving Argatroban, aspirin and Argatroban or 7E3-F(ab')2, a significant decrease in hematocrit was observed, but not in the aspirin alone group. Although hemodilution might have contributed to a reduced tendency for platelet deposition, the dissimilar effect of the various infusion protocols on the reocclusion rate argues against the hypothesis that this was the main or a primary mechanism for the observed phenomenon. The use of Argatroban alone or in combination with aspirin prolonged the bleeding time to slightly abnormal levels and prolonged both thrombin time and activated partial thromboplastin time, with return of the partial thromboplastin time toward normal within 2 h.

Role of heparin. We have previously shown (19) that combinations of heparin, 7E3-F(ab')2 and rt-PA accelerate lysis and completely prevent reocclusion in a canine model of myocardial infarction. However, in the present study, the reversal of the effect of heparin by protamine sulfate appeared to diminish the effect of 7E3-F(ab')2 and consistent prevention of reocclusion was not seen. This suggests an important role for thrombin inhibition in the maintenance of vascular patency, which is not completely blocked by saturation of the platelet glycoprotein IIb/IIIa receptors.

Potential clinical relevance. The persistence of coronary patency despite the return of bleeding time, thrombin time and partial thromboplastin time toward normal suggests that the duration of the bleeding time prolongation induced by antiplatelet therapy that is required to permit deactivation of the damaged endothelial surface may be relatively short.

Combination therapy with antiplatelet agents that affect different platelet activation pathways may permit a reduction in the toxicity of any single agent by lowering the dose required to inhibit platelet function. Thus, acceleration of lysis and prevention of reocclusion might be accomplished without or with less prolongation of the bleeding time and a consequent reduced risk of bleeding. Provided these concepts can be extrapolated to humans, combination therapy with rt-PA, antiplatelet agents and specific thrombin inhibitors might result in better efficacy to toxicity ratios for pharmacologic recanalization of occluded coronary arteries in patients with acute myocardial infarction.

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